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A Submaximal Dose of Insulin Promotes Net Skeletal Muscle Protein Synthesis in Patients With Severe Burns

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Objective
To investigate the hypothesis that a submaximal insulin dose reverses the net muscle catabolism associated with severe burns, and to determine its effects on amino acid kinetics.

Summary Background Data
The authors previously showed that a maximal dose of insulin administered to patients with severe burns promoted skeletal muscle glucose uptake and net protein synthesis. However, this treatment was associated with caloric overload resulting from the large quantities of exogenous glucose required to maintain euglycemia, and hypoglycemia was a potential problem.

Methods
Thirteen patients were studied after severe burn injury (>60% total body surface area). Patients were randomly treated by standard care (n = 5) or with exogenous insulin (n = 8). Data were derived from an arteriovenous model with primed-continuous infusions of stable isotopes and biopsies of the vastus lateralis muscle.

Results
Net amino acid balance was significantly improved with insulin treatment. Skeletal muscle protein synthesis was significantly greater in the group receiving insulin, whereas muscle protein breakdown was not different between the groups. This submaximal dose of insulin did not affect glucose or amino acid uptake or require a greater caloric intake to avoid hypoglycemia.

Conclusions
Submaximal insulin can promote muscle anabolism without eliciting a hypoglycemic response.

Severe burn injury causes a metabolic state characterized by an increase in skeletal muscle proteolysis that lasts for weeks and results in extensive loss of lean body mass.[1,2] The resultant debilitation from muscle loss compromises the patient's ability to handle complications during the hospital stay and encumbers rehabilitative efforts.[3] For these reasons, recent effort has focused on the amelioration of muscle catabolism by administration of anabolic agents.

The anabolic effects of insulin in normal volunteers has been repeatedly demonstrated.[4–6] Insulin has been shown to promote muscle anabolism in healthy volunteers by either stimulating protein synthesis[4,5] or inhibiting protein breakdown.[4,6] To test the hypothesis that insulin would produce a similar response in patients with burns, Sakurai et al[7] investigated the effects of a maximal dose of insulin on skeletal muscle protein metabolism. Net muscle protein catabolism was ameliorated because of a fourfold increase in protein synthesis, resulting in a synthetic rate that exceeded that of muscle protein breakdown.[7]

In our previous study, insulin was infused at approximately 7.5 mU/kg per minute, or about 32 units/hour for a 70-kg patient. This administration rate resulted in plasma insulin concentrations of approximately 900 μU/ml. Because of this maximal hyperinsulinemic stimulus, large amounts (twice the resting energy expenditure) of exogenous glucose were required to avoid hypoglycemia.[7] Whereas most of this glucose appeared to be taken up peripherally,[8] and liver fatty acid synthesis was not stimu-

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Table 1. PATIENT CHARACTERISTICS

Patient No.	Age	% TBSA Burned	% 3rd Degree Burn	% Leg Burned	Days Post-Burn of Study	Caloric Intake (kcal/kg · d)
Control Group						
1	27	85	20	65	18	46.0
2	53	55	55	0	32	55.3
3	31	73	67	85	27	52.9
4	40	50	40	0	8	59.4
5	14	61	61	40	35	66.1
Mean	33.0	64.8	48.6	38.0	24.0	55.9
SEM	6.5	6.3	8.4	17.1	4.9	3.4
Insulin Group						
1	27	85	20	65	25	41.9
2	28	70	68	60	6	43.5
3	37	50	3	15	7	51.7
4	12	70	16	90	9	61.0
5	31	73	67	85	40	84.7
6	39	95	95	100	35	75.2
7	40	50	40	0	21	56.9
8	17	55	50	90	16	76.4
Mean	28.9	68.5	44.9	63.1	19.9	61.4
SEM	3.6	5.8	11.0	13.1	4.5	5.7

TBSA = total body surface area.

lated,[9] providing insulin in pharmacologic amounts gives some reason for concern. In particular, administering such high doses of insulin requires constant clinical vigilance because of the possibility of hypoglycemia. Therefore, the purpose of this study was to determine if a submaximal dose of insulin would promote skeletal muscle anabolism in patients with severe burn injury without inducing significant hypoglycemia.

METHODS

Patients

Patients were admitted to the adult burn unit at the University of Texas Medical Branch within 48 hours of injury. Fluid resuscitation was provided as previously described.[10] Within 48 hours of admission, the burn wound was excised and subsequently grafted with autograft or cadaveric allograft. Patients then typically returned to the operating room for reharvesting of donor sites every 7 to 10 days. The present studies were conducted the day before a follow-up surgery (22 ± 13 (SD) days after the burn). Patient data and injury characteristics are summarized in Table 1. Thirteen patients (12 male, 1 female) were studied after severe burn injury (>60% total body surface area). Patients were randomized into 2 treatment groups; control or standard care (n = 5) and exogenous insulin at an average rate of 2.6 mU/kg per minute (n = 8).

Each patient was studied during enteral feeding with Vivonex TEN (Sandoz Nutrition Corp., Minneapolis, MN). Vivonex contains 300 kcal/serving in the following caloric breakdown: 82.3% carbohydrate, 15% protein, 2.7% fat. Patients were given 45 kcal/kg of Vivonex plus an additional 25 kcal/kg for each percentage point of total body surface area burned. Written consent was obtained on all patients, and this protocol was approved by the Institutional Review Board at our institution.

Experimental Protocol

To stabilize the effects of exogenous insulin on injured patients,[11] insulin was infused at least 2 days before metabolic study. Table 2 delineates insulin treatment and response. Before the beginning of each metabolic study, a 3 French, 8-cm polyethylene catheter (Cook, Inc., Bloomington, IN) was inserted into the femoral vein and femoral artery under local anesthesia. Both femoral catheters were used for blood sampling; the femoral arterial catheter was also used for indocyanine green infusion for determining leg blood flow. A preexisting triple-lumen central venous catheter was used for stable isotope infusion and for measuring systemic concentration of indocyanine green. Baseline blood samples were obtained for the measurement of background amino acid enrichment and indocyanine green concentration.

Infusion studies were conducted as depicted in Figure 1. Stable isotope tracers were infused at the following primed (PD) continuous infusion rates (IR) throughout the 5-hour study: L-[ring-$^{2}H_{5}$]phenylalanine, IR = 0.07 μmol/kg · min, PD =2 μmol/kg; L-[1-^{13}C]leucine, IR = 0.08 μmol/kg · min, PD =4.8 μmol/kg; and L-[1-^{13}C]alanine, IR = 0.35

Table 2. EXOGENOUS INFUSION RATES AND PLASMA CONCENTRATIONS

Patient No.	Insulin Infusion Rate* (mU/kg · min)	Days of Insulin Infusion	Plasma Insulin (μU/ml)	Plasma Glucose (mg/dl)	Exogenous Glucose IR† (μmol/kg · min)
Control Group					
1	0	0	27.6	217	0
2	0	0	31.9	147	0
3	0	0	35.5	150	0
4	0	0	25.5	260	0
5	0	0	16.9	123	0
Mean			27.5	179.4	
SEM			3.2	25.5	
Insulin Group					
1	1.45	2	158.8	116	0
2	3.31	2	506.9	253	0
3	2.87	2	377.8	154	0
4	2.07	4	251.8	228	0
5	2.31	4	153.1	118	29.8
6	3.23	5	314.5	211	19.6
7	2.89	5	119.1	157	20.3
8	3.03	4	142	145	5.8
Mean	2.61	3.6	241.9	172.4	18.9 (9.4)‡
SEM	0.24	0.4	44.5	17.3	4.9 (4.2)

* Actual infusion rate.
† 25% dextrose.
‡ Mean and SEM of patients 5–8 only, values for all patients in parentheses.

μmol/kg · min, PD = 35 μmol/kg. Biopsies of the vastus lateralis were performed as previously described[12] at 2 and 5 hours of tracer infusion. The fractional synthetic rate of skeletal muscle protein was determined by the incorporation of L-[ring-$^2H_5$]phenylalanine into protein from 2 to 5 hours.

Arteriovenous blood samples were drawn at 20-minute intervals between hours 4 and 5 to determine amino acid kinetics. In addition, leg blood flow was determined by indocyanine green infusion during this sampling hour. To measure leg blood flow, a continuous infusion (IR = 0.5 mg/min) of indocyanine green was started 15 minutes before the sampling hour. Subsequent sampling was performed simultaneously from the femoral vein and the central vein over the hour. Arterial samples for amino acid kinetics were always taken after those from the femoral and central veins to avoid interference with blood flow measurement. After each sampling, indocyanine green infusion was uninterrupted for at least 10 to 15 minutes before the next blood flow measurement.

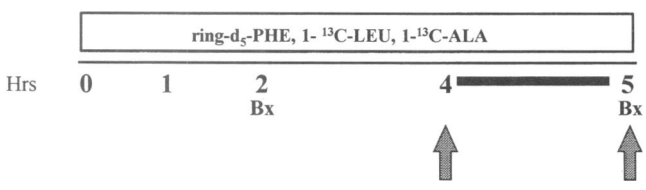

Figure 1. Stable isotope infusion protocol. Solid bar indicates arteriovenous sampling and blood flow determination; arrows indicate arteriovenous amino acid determination. Bx = muscle biopsy.

For the patients receiving exogenous glucose, blood glucose was sampled every hour during the course of clinical care. If blood glucose concentrations fell to <100 mg/dl, exogenous dextrose was infused in an effort to maintain blood glucose concentrations between 120 and 200 mg/dl. Plasma lactate and glucose concentrations were measured from the femoral artery and vein with each sampling from 4 to 5 hours. Lactate and glucose levels were determined simultaneously on a Glucose/Lactate 2300 Stat Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) with the average of four values reported as the concentration over the sampling hour.

Analysis of Samples

Blood

The blood concentrations of unlabeled phenylalanine, leucine, and alanine, as well as the enrichment of their isotopic counterparts, were simultaneously determined by gas chromatography–mass spectrometry (GCMS) using the internal standard approach and the nitrogen-acetyl-*n*-propyl esters, as previously described.[13] The isotopic enrichment of free amino acids in blood was determined on an HP Model 5989 GCMS (Hewlett-Packard Co., Palo Alto, CA) by chemical ionization and selected ion monitoring.[14]

Muscle

Tissue biopsies of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then

stored at −80°C until processed. The tert-butyldimethylsilyl (TBDMS) derivative was prepared for the intracellular free water as previously described[15] and analyzed by GCMS using electron impact ionization. The protein-bound enrichment of phenylalanine was analyzed as previously described[16,17] by GCMS.

Plasma Insulin

Blood was drawn from the femoral vein at the beginning and end (5 hours) of the isotope infusion study to determine insulin concentrations. Serum insulin concentrations were then determined by commercial radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), and the average of the two values was reported (see Table 2).

Plasma Amino Acid Concentrations

Arterial and venous amino acid concentrations were determined in heparinized plasma at 4 and 5 hours of the isotope infusion study (see Fig. 1). Free amino acid concentrations were determined by high-performance liquid chromatography, as previously described.[18]

Calculations

Kinetic Model

Leg amino acid kinetics were calculated according to a three-pool compartment model that has been derived[19] and presented[5,12,15] previously. The kinetics of free amino acids in leg skeletal muscle are described by the model in Figure 2. The three compartment model parameters were calculated as follows:

$$F_{in} = C_A \cdot BF$$

$$F_{out} = C_V \cdot BF$$

$$F_{M,A} = ([(E_M - E_V)/(E_A - E_M)] \cdot C_V + C_A) \cdot BF$$

$$F_{V,M} = ([(E_M - E_V)/(E_A - E_M)] \cdot C_V + C_V) \cdot BF$$

$$F_{V,A} = F_{in} - F_{M,A}$$

$$F_{M,O} = F_{M,A} \cdot (E_A/E_M - 1)$$

$$F_{O,M} = (C_A \cdot E_A - C_V \cdot E_V)\ BF/E_M$$

$$Ra_M = F_{M,O} + F_{M,A}$$

where C_A and C_V are the blood free amino acid concentrations in the femoral artery and vein, respectively; E_A, E_V, and E_M are amino acid enrichments (tracer/tracee ratio) in the femoral artery, vein, and muscle, respectively; and BF is leg blood flow.

Fractional Synthetic Rate of Muscle Protein

Skeletal muscle fractional synthetic rate was calculated from the determination of the rate of tracer incorporation into the protein and the enrichment of the intracellular pool as the precursor by the following equation, as previously described:[16]

$$FSR = [(E_{p2} - E_{p1})/E_M \cdot t)] \cdot 60 \cdot 100$$

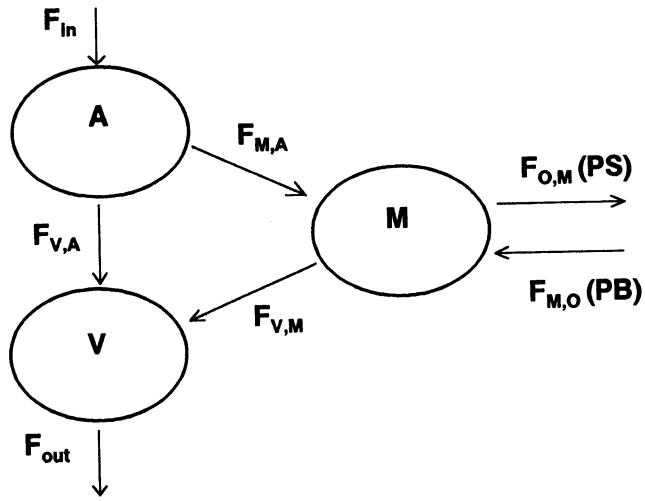

Figure 2. Three-compartment model of leg amino acid kinetics. Free amino acid pools in the femoral artery (A), femoral vein (V), and muscle (M) are connected by arrows indicating unidirectional flow between each compartment. Amino acids enter the leg via the femoral artery (F_{in}) and leave via the femoral vein (F_{out}). $F_{V,A}$, direct amino acid flow from artery to vein without entering intracellular fluid; $F_{M,A}$ and $F_{V,M}$, inward and outward amino acid transport from artery to muscle and from muscle to vein, respectively; $F_{M,O}$, intracellular amino acid appearance from endogenous sources (*i.e.*, proteolysis and *do novo* synthesis, if any); $F_{O,M}$, rate of disappearance of intracellular amino acids (*i.e.*, protein synthesis and other metabolic fates, if any).

Data Presentation and Statistical Analysis

Data are presented as means ± SEM. Control patients who did not receive exogenous insulin were then compared with insulin patients by t test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Effect of Insulin Infusion

Patients received an average of 2.6 mU/kg per minute (range 1.45 to 3.31; see Table 2). Insulin infusion at a lower rate was associated with a blunted response. Three patients received exogenous insulin at an average rate of 1.7 mU/kg per minute (range 1.55 to 1.82) but responded with plasma insulin concentrations of only 63 ± 5 μU/ml. Because the mean insulin concentration of these 3 patients fell outside the 95% confidence interval of insulin-infused patients, they were eliminated from data presentation. Individual patient response to insulin infusion varied and was not directly related to the infusion rate (see Table 2). However, an average insulin infusion rate of 2.61 ± 0.24 mU/kg per minute in 8 patients studied resulted in a mean plasma

Table 3. LEG SUBSTRATE BALANCE

	Control	Insulin
Arteriovenous glucose (μmol/ml)	0.28 ± 0.05	0.31 ± 0.06
Glucose clearance (l/min · 100 ml leg)	0.024 ± 0.006	0.040 ± 0.012
Arteriovenous lactate (mmol/L)	−0.1 ± 0.01	−0.2 ± 0.01
Phenylalanine net balance (nmol/min · 100 ml leg)	−62 ± 20	−15 ± 13*
Leucine net balance (nmol/min · 100 ml leg)	−53 ± 30	37 ± 9*
Alanine net balance (nmol/min · 100 ml leg)	−251 ± 135	−336 ± 110
Arteriovenous total nitrogen† (μmol/min · 100 ml leg)	−2.1 ± 0.7	1.6 ± 1.4*
Arteriovenous essential nitrogen† (μmol/min · 100 ml leg)	−0.2 ± 0.2	0.8 ± 0.5‡
Arteriovenous Nonessential nitrogen† (μmol/min · 100 ml leg)	−1.9 ± 0.5	0.9 ± 1.0*

* $p < 0.05$ versus control.
† $p = 0.07$ versus control.
‡ Average of 4- and 5-hour samples.

insulin concentration of 241.9 ± 44.5 μU/ml. The control group had significantly lower plasma insulin concentrations (27.5 ± 3.2 μU/ml, $p < 0.05$).

Four of the eight patients studied required exogenous glucose to prevent the blood glucose concentrations from dropping to <120 mg/dl (see Table 2). For these 4 patients, the average requirement was 14 g/hour for a 70-kg patient, or about 46 cc/hour of 25% dextrose. However, total carbohydrate intake did not vary between groups (46.2 ± 2.8 kcal/kg control group *vs.* 50.2 ± 4.9 kcal/kg insulin group). Venous plasma glucose concentrations were also not different between the groups (179.4 ± 25.5 mg/dl control group *vs.* 172.4 ± 17.3 mg/dl insulin group).

Substrate Balance

Glucose, lactate, and amino acid balance across the leg are shown in Table 3. Glucose and lactate arteriovenous balances were not different between groups. In addition, glucose clearance was not significantly different with insulin treatment. Phenylalanine and leucine net balance displayed a significantly greater muscle uptake ($p < 0.05$) with insulin. In the insulin group, phenylalanine net balance was not significantly different than zero; leucine net balance was significantly greater than zero. The arteriovenous difference in nitrogen from nonessential amino acids was significantly greater with insulin treatment ($p < 0.05$), with the difference in essential nitrogen approaching significance ($p = 0.07$).

Amino Acid Kinetics

Tissue transport was unaffected by insulin infusion. Inward ($F_{M,A}$) and outward ($F_{V,M}$) transport were not different between groups for any of the three amino acid tracers. Figure 3 demonstrates that model-derived values of protein synthesis ($F_{O,M}$) as calculated by phenylalanine tracer were significantly greater in the insulin group, whereas protein breakdown ($F_{M,O}$) was not different between the groups. Thus, the net protein balance across the leg was significantly greater in the insulin group (see Table 3 and Fig. 3). Cal-

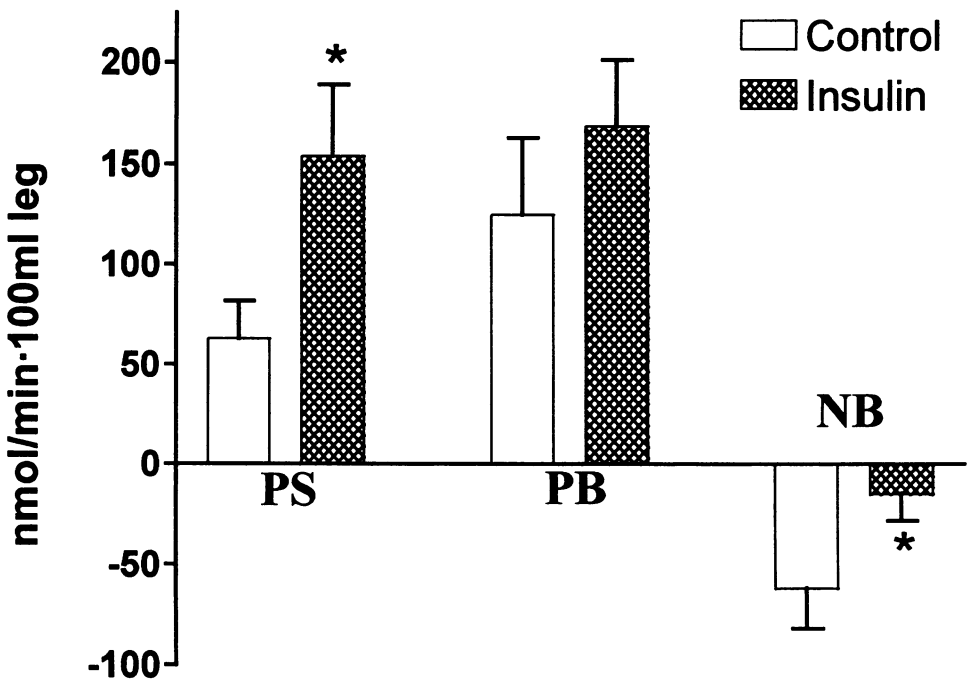

Figure 3. Model calculations of protein synthesis (PS; $F_{O,M}$) and protein breakdown (PB; $F_{M,O}$) by phenylalanine tracer, and net phenylalanine balance (NB). *, $p < 0.05$ *vs.* controls. NB is not significantly different than zero.

culation of fractional synthetic rate by direct incorporation of L-[ring-$^2H_5$]phenylalanine into skeletal muscle protein independently confirms an increase in protein synthesis (0.309 ± 0.037% per hour insulin group *vs.* 0.160 ± 0.021% per hour control group; $p < 0.05$).

Leucine tracer calculation of protein synthesis ($F_{O,M}$) was also significantly greater in the insulin group (347 ± 64 nmol/min per 100 ml of leg *vs.* 131 ± 25; $p < 0.02$). Calculation of protein breakdown ($F_{M,O}$), as with phenylalanine, was not different between the groups, again resulting in a significantly greater net leucine balance across the leg (see Table 3). All other kinetic parameters for each tracer, including *de novo* synthesis and intracellular appearance (Ra_M) of alanine, were not significantly different between the groups.

DISCUSSION

This study demonstrates that submaximal insulin administration in severely burned patients can enhance skeletal muscle protein anabolism. With the present mean administration rate (≈2.6 mU/kg per minute), insulin's effects on skeletal muscle were primarily through an increase in muscle protein synthesis, without a concomitant increase in protein breakdown or amino acid transport. Our study also demonstrates that lower-dose insulin administration can promote muscle anabolism without the need for increased caloric intake. Although the quantitative increase in protein synthesis and leg amino acid net balance is striking, as in our previous investigation,[7] there are notable differences between the two studies concerning maximal and submaximal insulin administration. This distinction centers on the hypoglycemic *versus* protein-related actions of the different doses of insulin.

Sakurai et al[7] demonstrated that a pharmacologic dose of insulin markedly increased glucose uptake into leg tissue. Glucose uptake was accompanied by an increase in amino acid uptake and increased lactate release from the leg. The present study indicates that insulin administered at approximately one-third the previous rate had a minimal effect on glucose uptake or clearance (see Table 3) in skeletal muscle. Alanine kinetics support this finding: insulin did not affect the *de novo* synthesis of alanine, which would be expected to be increased if glucose uptake were increased.[20] This study also demonstrates that submaximal insulin has no effect on amino acid transport/uptake in skeletal muscle; rather, the amino acid precursors for the accelerated protein synthesis came from a more efficient reutilization of amino acids from protein breakdown. The relation between plasma insulin and glucose uptake is established in burned patients. The maximal physiologic effectiveness of insulin on glucose uptake is not significantly reduced in the burned patient compared with the bed-rested control.[21] However, within the physiologic range of insulin concentration, the hypoglycemic action of insulin is blunted in the burned patient.[22] The present study further supports the observations that maximal doses are required to stimulate glucose uptake into skeletal muscle.

The failure of insulin to stimulate glucose uptake at concentrations that are normally effective, while at the same time stimulating muscle protein synthesis, implies that the deficiency in the hypoglycemic action of insulin is not mediated at the receptor level. Insulin must bind to its receptor, regardless of whether its ultimate intracellular action is on glucose or protein metabolism. The responsiveness of protein metabolism to submaximal doses of insulin implies that insulin was properly binding to its receptor. Thus, the impairment in the normal hypoglycemic action of insulin most likely results from a deficiency in the postreceptor signaling, which leads normally to an increase in glucose uptake.

Contrary to the characteristics of glucose metabolism, protein synthesis can be stimulated at submaximal insulin concentrations. An increase in protein synthesis was reflected in model calculations ($F_{O,M}$) involving both essential amino acid tracers, phenylalanine and leucine, and the independent measure of direct incorporation of the phenylalanine tracer (fractional synthetic rate). Although leucine can be oxidized in the muscle, the increase in calculated protein synthesis and the resultant increased net balance are most likely the result of increased incorporation into protein, because hyperinsulinemia is known to inhibit leucine oxidation.[23] Because protein breakdown was unaffected at the present insulin concentrations, net balance across the leg of both phenylalanine and leucine improved significantly.

The mechanisms accompanying stimulation of protein synthesis differ between maximal and submaximal dosages. Stimulation of protein synthesis with a maximal dose was accompanied by an increase in amino acid uptake and protein breakdown.[7] In the present study, protein synthesis was stimulated without a concomitant increase in amino acid uptake or protein breakdown. In the prior study, calculated protein synthesis was stimulated to a greater degree than in our present study. Despite the fact that protein breakdown in a fed burned patient is more than twice that of a fasted, healthy subject,[12] a further increase was required at maximal insulin to maintain the intracellular pool of amino acids required for such an increase in protein synthesis.[7] Because there is a demonstrated relation between protein synthesis and amino acid availability,[13,24] the amino acid demand of protein synthesis was also accompanied by an increase in inward transport, because breakdown was not sufficient to provide all the needed precursors for synthesis.[7] In the present study, the amino acid demands of a lower absolute rate of protein synthesis could be met by the existing (high) rate of protein breakdown. As a result, there was no intracellular requirement for an increase in protein breakdown or inward amino acid transport. The demonstrated increase in arteriovenous balance of nitrogen (see Table 3) of the insulin group supports this concept. Intracellular amino acids derived from protein breakdown were

reused for protein synthesis rather than released from the muscle.

It is reasonable to suggest that skeletal muscle and lean body mass could be maintained throughout acute care. Calculation of amino acid net balance by phenylalanine was not different than zero, whereas leucine net balance was significantly greater than zero (see Table 3). Thus, the traditional net efflux of amino acids from the muscle after burn injury[25] can be ameliorated by submaximal insulin administration. Most importantly, this lower insulin dose greatly reduces the requirement for excess glucose (and caloric) intake to prevent hypoglycemia (see Table 2).

In this severely burned population, there is a weak relation between a given insulin infusion rate and plasma insulin concentrations. Apparently, a patient's ability to metabolize or clear insulin varies considerably. This was especially true at the lower insulin infusion rates, where patients' response were not representative of insulin-infused patients. Although the mean infusion rate of approximately 2.6 mU/kg per minute in this study results in a mean plasma insulin concentration of 242 μU/ml, the actual insulin concentration during this rate of insulin infusion may vary widely. In addition, the plasma glucose concentrations maintained in this study may not be ideal. In our previous study, plasma glucose was maintained at approximately 115 mg/dl,[7] a concentration that enabled glucose uptake by the muscle[8] without exacerbating hepatic triglyceride production.[9] Although not tested directly, the higher plasma glucose concentrations in the present study may not have the same desired effect on liver glucose uptake and fatty acid synthesis. This is because hepatic glucose uptake is dependent on the plasma glucose concentration and is not controlled by insulin. Glucose clearance across the leg tended to increase, but this increase was not significant and therefore does not preclude an increased uptake by the liver. The concern of glucosuria and subsequent osmotic diuresis also exists at higher plasma glucose concentrations.

Although the current level of insulin administration provides some encouraging results and advantages over the previous maximal dose, clinical vigilance is still required. Future use of exogenous insulin should concentrate on the control of hyperglycemia, with plasma glucose concentrations maintained closer to 115 mg/dl. Also, it would be useful to assess a patient's response to insulin infusion by analysis of the plasma insulin concentration, which is not a routine clinical laboratory analysis. However, insulin administration in this submaximal range would be expected to increase net protein synthesis if the effects on plasma glucose were apparent. The amount of insulin used in this study equates to approximately 10 to 12 units/hour for a 70-kg patient.

In conclusion, this study provides evidence that an inexpensive modality, submaximal insulin administration, promotes muscle anabolism in severely burned patients. Submaximal insulin can promote net muscle protein synthesis while minimizing hypercaloric requirements. Net muscle protein synthesis is achieved with normal feedings by efficient reuse of intracellular amino acids. Treatment throughout the entire course of acute care would be the next logical step to ascertain the overall clinical relevance of insulin's anabolic action on skeletal muscle.

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